Consumer and Corporate Affairs Canada
Consommation et Corporations Canada

(11) (A) No. 1 232 837

(45) ISSUED 880216

(52) CLASS 167-35
C.R. CL. 167-46

(51) INT. CL.[4] A61K 49/00

(19) (CA) **CANADIAN PATENT** (12)

(54) Ultrasound Contrast Agent Containing Microparticles and Gas Micro-Bubbles

(72) Hilmann, Jürgen;
Lange, Lothar;
Zimmermann, Ingfried,
Germany (Federal Republic of)

(73) Granted to Schering Aktiengesellschaft
Germany (Federal Republic of)

(21) APPLICATION No. 451,729

(22) FILED 840411

(30) PRIORITY DATE Germany (Federal Republic of)
(P 33 13 947.4) 830415

No. OF CLAIMS 37 - NO-DRAWING

Canada

The invention relates to contrast agents for use in ultrasound diagnosis of the human or animal body.

The examination of organs using ultrasound (sonography) is a diagnostic method which has been well established and practised for some years. Ultrasound waves in the megahertz range (above 2 megahertz with wavelengths of between 1 and 0.2 mm) are reflected at the interfaces of various types of tissue. The resulting echoes are amplified and rendered visible. Of particular importance is the examination of the heart by this method which is known as echocardiography (Haft, J.I. et al.: Clinical echocardiography, Futura, Mount Kisco, New York 1978; Kohler, E. Klinische Echokardiographie, Enke, Stuttgart 1979; Stefan, G. et al.: Echokardiographie, Thieme, Stuttgart-New York 1981; G. Biamino, L. Lange: Echokardiographie, Hoechst AG, 1983.).

Since fluids, including blood, produce ultrasound image contrast only when there are differences in density with respect to the surroundings, possibilities were sought of rendering the blood and its circulation visible for ultrasound examination and this may be effected by injecting extremely fine gas bubbles into

the bloodstream.

Several methods of producing and stabilising gas bubbles have been described in the literature. They can be produced, for example, before injection into the bloodstream, by vigorously shaking or stirring a liquid solution, such, for example, as sodium chloride solution, dye solution or previously removed blood.

Although ultrasound image contrast is achieved by these methods, they have serious disadvantages which are manifested in poor reproducibility, greatly fluctuating size of the gas bubbles and a certain risk of embolism due to a proportion of large visible bubbles. Some of these disadvantages have been eliminated by other production processes, such as, for example, by the process of U.S. Patent No. 3,640,271 in which bubbles of a reproducible size are produced by filtration or by the use of direct current electrode apparatus. Against the advantage of being able to produce gas bubbles of a reproducible size is the disadvantage of the considerable technical outlay involved.

U.S. Patent No. 4,276,885 describes the production of gas micro-bubbles of a specific size which are surrounded by a gelatine membrane which protects them from coalescence. The prepared bubbles can be stored only in the "frozen" state, for example by storing at refrigerator temperature, and they must

be raised to body temperature again before they can be used.

U.S. Patent No. 4,265,251 describes the production and use of gas micro-bubbles with a solid saccharide covering, which bubbles may be filled with a pressurised gas. If they are under normal pressure, they can be used as ultrasound image contrast agents; when used at an elevated internal pressure, they can be used for measuring blood pressure. Although in this case the storage of the solid gas bubbles does not present any problem, the technical outlay involved in their production gives rise to high costs as a result of the complex techniques.

The risks involved with the hitherto known contrast agents for ultrasound diagnosis are caused by two factors: the size and number both of the particles of solid material and also of the gas bubbles.

The ultrasound contrast agents prepared by the previously described methods have, in all cases, possessed only some of the following properties that are required:-

1. Exclusion of the risk of embolism (dependent on size and number of gas bubbles and size and number of particles of solid material).
2. Reproducibility.
3. Sufficiently long stability.

4. Ability to pass through the lungs, for example in order to obtain ultrasound image contrast of the left-hand side of the heart.

5. Ability to pass through the capillaries.

6. Sterility and freedom from pyrogens.

7. Easy production at reasonable cost.

8. Easy storage.

European Patent Application No. 52575 published May 26, 1982 to Ultra Med. Inc. describes the production of ultrasound contrast agents containing gas bubbles that are supposed to possess these necessary properties. However, in order to produce them, microparticles of a solid crystalline substance, such as, for example, galactose, are suspended in a liquid carrier, and the gas, which is adsorbed at the particle surface and is enclosed in cavities between the particles or in intercrystalline cavities, forms the gas bubbles. The resulting suspension of gas bubbles and microparticles is injected over a period of 10 minutes. Although according to European Patent Specification 52575 the suspension prepared by the described method is capable, after injection into a peripheral vein, of appearing both on the right-hand side of the heart and also, after passing through the lungs, on the left-hand side of the heart and of rendering visible the blood there and its circulation during ultrasound examination, it was found when checked that the contrast medium prepared by the method

X

described in European Application No. 52575 and injected into a peripheral vein did not in fact produce ultrasound echoes in the left-hand side of the heart.

An object of the present invention is to provide a contrast agent for ultrasound diagnosis which is capable, after being administered intravenously, of rendering visible for ultrasound the blood and its circulation conditions not only on the right-hand side of the heart but also, after passing through the capillary bed of the lungs, on the left-hand side of the heart. In addition, it should also permit the representation of the circulation of blood through other organs, such as the myocardium, the liver, the spleen and the kidneys.

The present invention provides a contrast agent for use in the ultrasound diagnosis of the human or animal body, which comprises, in a liquid carrier, a mixture of microparticles of a semi-solid or liquid surface-active substance and microparticles of a solid non-surface-active substance and micro-bubbles of a gas.

It will be understood that the constituents of the contrast agents of the invention must be physiologically tolerable, and this, of course, equally applies to the liquid media and diagnostic kits described below.

The invention also provides a liquid medium for

use in making up the ultrasound contrast agent, which comprises a suspension of a mixture of microparticles of a semi-solid or liquid surface-active substance and microparticles of a solid non-surface-active substance in a liquid carrier.

The ultrasound contrast agents of the invention possess all the above-mentioned properties that are expected of such a contrast agent.

Surprisingly, we have found that by suspending a mixture of microparticles of a semi-solid or liquid surface-active substance and microparticles of a solid non-surface-active substance in a liquid carrier, an ultrasound contrast agent is obtained which, after being injected into a peripheral vein, permits reproducible ultrasound images even of blood in the arterial left-hand side of the heart. Since the left-hand side of the heart can be reached with the ultrasound contrast agent of the invention after intravenous administration, ultrasound contrasts of other organs supplied with blood from the aorta, such as the myocardium, the liver, the spleen, the kidneys, inter alia, are therefore also possible after venous administration. The ultrasound contrast agent of the invention is, of course, also suitable for contrasts on the right-hand side of the heart and for all other uses as an ultrasound image contrast medium.

All substances that are physiologically tolerable

in the quantities used, that is, that have a low toxicity and/or are biologically degradable and the melting point of which is lower than room temperature, that is to say those that are semi-solid or liquid at room temperature, are suitable as the semi-solid or liquid surface-active substances which are a constituent of the mixture with a non-surface active solid substance that is used for the production of the microparticles. Especially suitable are lecithins, lecithin fractions and their conversion products, polyoxyethylene fatty acid esters, polyoxyethylene fatty alcohol ethers, polyoxyethylated sorbitan fatty acid esters, glycerine polyethylene glycol oxystearate, glycerine polyethylene glycol ricinoleate, ethoxylated soya sterols, ethoxylated castor oils and their hydrogenated derivatives, polyoxyethylene fatty acid stearates and polyoxyethylenepolyoxypropylene polymers, saccharose esters, or saccharose glycerides and xyloglycerides such, for example, as soya oil saccharose glyceride and palm oil xylite, unsaturated (C_4-C_{20})-fatty alcohols or (C_4-C_{20})- fatty acids, mono-, di- and tri-glycerides, fatty acid esters of saccharose or fatty acid esters such, for example, as butyl stearate, palm oil saccharose glyceride or cotton seed oil saccharose glyceride; butyl stearate, soya oil saccharose glyceride and polyethylene glycol sorbitan

monostearate are preferred.

The rate at which the microparticles of the surface-active substance dissolve in the liquid carrier should be slower than the rate at which these microparticles dissolve in the blood. Advantageously, the solubility of the microparticles of the surface-active substance in the liquid carrier is such that when they are introduced into it they do not start to dissolve in it to a substantial extent for at least 10 minutes. It will be appreciated that upon administration of the contrast agent the microparticles of the surface-active substance will start to dissolve in the blood.

The semi-solid or liquid surface-active substance is used in a concentration of from 0.01 to 5 % by weight, preferably from 0.04 to 0.5 % by weight.

As solid non-surface-active substances there come into consideration organic and inorganic compounds, for example salts such, for example, as sodium chloride, sodium citrate, sodium acetate or sodium tartrate; monosaccharides such, for example, as glucose, fructose or galactose; disaccharides such, for example, as saccharose, lactose or maltose; pentoses such, for example, as arabinose, xylose or ribose; or cyclodextrines such, for example, as α-, β- or γ-cyclodextrine; galactose, lactose and α-cyclodextrine are preferred. They are contained in the contrast agent of the invention in a concentration of from 5 to 50 % by

weight, preferably from 9 to 40 % by weight.

The microparticles may be produced by recyrstallising the non-surface-active substance under sterile conditions. Subsequently, under sterile conditions, the surface-active substance is mixed with the non-surface-active solid substance and comminuted, for example by grinding in an air-jet mill, until the desired particle size is obtained. Preferably the microparticles should have a median particle size of less than 10 µm, advantageously less then 8 µm, more especially within the range of from 1 to 3 µm. The particle size is determined in a suitable measuring apparatus. The ratio by weight of surface-active substance to non-surface-active substance is preferably from 0.01 to 5:100.

Both the microparticle size achieved by the comminution process and also the size of the micro-bubbles containing a physiologically tolerable gas in the contrast agent of the invention ensure safe passage through the capillary system and the capillary bed of the lungs and preclude the occurrence of embolism.

Some of the micro-bubbles required to produce image contrast are transported by the suspended micro-particles, adsorbed at the surface of the microparticles and enclosed in the cavities between the microparticles or enclosed in an intercrystalline manner.

The volume of physiologically tolerable gas transported by the micro-particles in the form of

micro-gas bubbles is from 0.02 to 0.6 ml per gram of microparticles.

Apart from its transporting function, the carrier liquid has the task of stabilising the suspension comprising microparticles and gaseous micro-bubbles, for example of preventing the sedimentation of the microparticles and the coalescing of the micro-bubbles or of delaying the dissolving process of the microparticles.

There may be used as the liquid carrier, for example, water, aqueous solutions of one or more inorganic salts such, for example, as physiological sodium chloride solution and buffer solutions, aqueous solutions of mono- or di-saccharides such, for example, as galactose, glucose or lactose, monohydric or polyhydric alcohols, in so far as they are physiologically tolerable such, for example, as ethanol, propanol, isopropyl alcohol, polyethylene glycol, ethylene glycol, glycerine, propylene glycol, propylene glycol methyl ester or their aqueous solutions.

Water and physiological electrolyte solutions, such, for example, as physiological sodium chloride solution, and aqueous solutions of galactose and glucose, are preferred. If solutions are used, the concentration of the dissolved substance should be from 0.1 to 30 % by weight, preferably from 0.5 to 25 % by weight, and, more especially there may be mentioned, 0.9 % aqueous sodium chloride solution or 20 % aqueous galactose solution.

The invention also provides a process for the preparation of the contrast agent of the invention, wherein a mixture of microparticles of a semi-solid or liquid surface-active substance and microparticles of a solid non-surface-active substance, are mixed with a liquid carrier and agitated until a homogeneous suspension is formed.

In order to prepare the ultrasound contrast agent in a form ready for use, the sterile carrier liquid may be added to the microparticles of a mixture of a semi-solid or liquid surface-active substance and a solid non-surface-active substance, and this mixture with the liquid carrier is agitated until a homogeneous suspension has formed, which takes approximately from 5 to 10 secpnds. Immediately after its preparation, and at the later up to 5 minutes thereafter, the resulting suspension is injected in the form of a bolus into a peripheral vein or into a catheter which is already present, from 0.01 ml to 1 ml/kg body weight being administered.

For reasons of expediency, the components necessary for the preparation of the contrast agent of the invention such, for example, as carrier liquid and the mixture of microparticles of a semi-solid or liquid surface-active substance and the microparticles of the solid non-surface-active substance are stored under sterile conditions in two separate vessels (A) and (B).

respectively in the quantity necessary to carry out an examination. The size of vessel (B) should be such that the contents of vessel (A) can be transferred to (B) by means of an injection syringe and the combined components can be shaken.

The present invention also provides a diagnostic kit for use in the ultrasound diagnosis of the human or animal body, which comprises

(A) a container which contains a liquid carrier, and

(B) a second container which contains a mixture of microparticles of a semi-solid or liquid surface-active substance and microparticles of a solid non-surface-active substance.

The contents of the containers are in a form ready for mixing together immediately before use.

Preferably container (A) is provided with a closure permitting the removal of the contents under sterile conditions and container (B) is provided with a closure permitting, under sterile conditions, the addition of the contents of vessel (A) and the removal of the resulting contrast agent.

Advantageously the containers A and B both have a volume of from 5 to 10 ml. Preferably the ratio by weight of the microparticles of the surface-active substance to the microparticles of the non-surface-active substance is from 0.01 to 5:100.

The use of a contrast agent of the invention is demonstrated by an echocardiographic examination of a baboon weighing 10 kg which will now be described.

5 ml of carrier liquid (prepared according to Example 1 A below) are removed from a phial using an injection syringe and are added to 2 g of microparticles (prepared according to Example 1 B below) which are in a second phial, and the mixture is shaken for approximately from 5 to 10 seconds until a homogeneous suspension has formed. 2 ml of this suspension are injected into a peripheral vein (V. jugularis, brachialis or saphena) via a three-way tap having an infusion speed of at least 1 ml/sec., preferably 2-3 ml/sec. Immediately after injecting the contrast agent, 10 ml of physiological sodium chloride solution are injected at the same speed so that the contrast agent bolus is maintained as complete as possible until the right-hand side of the heart is reached. Before, during and after injection, a commercially available transducer for echocardiography is held against the thorax of the experimental animal so that a typical cross-section is obtained through the right-hand side and the left-hand side of the heart. This test procedure is understood and well known to a person skilled in the art.

If the ultrasound contrast agent reaches the right-hand side of the heart, it is possible to follow in a 2-D echo image or an M-mode echo image how the blood

marked by the contrast agent first reaches the level of the right-hand atrium and then the level of the right-hand ventricle and the pulmonary artery, homogeneous filling occurring for approximately 10 seconds. While the cavities in the right-hand side of the heart in the ultrasound image empty again, the blood which is rendered visible with contrast agent, after passing through the lungs, appears again in the pulmonary veins and fills the left atrium, the left ventricle and the aorta homogeneously, the contrast lasting from 2 to 3 times longer than on the right-hand side of the heart. In addition to the representation of the blood flow through the cavities of the left-hand side of the heart, there is also a representation of the myocardium showing the circulation of the blood.

The use of the ultrasound contrast agent of the invention is, however, not limited to rendering visible the circulation of blood in the arterial part of the heart after venous administration but is also used with outstanding success as a contrast agent for examining the right-hand side of the heart and other organs.

The invention still further provides a method of ultrasound diagnosis of the human or animal body, wherein a contrast agent of the invention containing a dispersion of micro-bubbles is injected into a part of the human or animal body, preferably intravascularly, and an ultrasound image of the micro-bubbles at a site in the body which it is desired to investigate is obtained.

The following Examples illustrate the invention, the parts and percentages being by weight unless otherwise indicated.

Example 1

A) Preparation of the carrier liquid:

5 ml phials are each filled with 4 ml of water used for injection purposes and sterilised for 20 minutes at 120°C.

B) Preparation of the microparticles:

A solution, filtered under sterile conditions, of 0.5 g of butyl stearate in 40 g of isopropanol is absorbed under sterile conditions on 199.5 g of sterile galactose particles, the isopropanol is removed by drying at 40°C and 200 torr, and the particles are then ground in an air-jet mill until the following size distribution of the particle size is obtained:

Median value: 1.9 µm

at least 99 % $<$ 6 µm

at least 90 % $<$ 3 µm.

The particle size and the distribution thereof are determined in a particle-measuring apparatus, for example after suspension in isopropanol. 5 ml phials are each filled with 2 g of the microparticles.

C) For the preparation of 5 ml of the ultrasound contrast agent in a form ready for use, the contents of the phial containing carrier liquid (water for injection purposes, A) are introduced by means of an injection syringe into the phial containing microparticles (B) and shaken until a homogeneous suspension is formed (from 5 to 10 seconds).

Example 2

A) Preparation of the carrier liquid:

5 ml phials are each filled with 4 ml of water used for injection purposes and sterilised for 20 minutes at 120°C.

B) Preparation of the microparticles:

A solution, filtered under sterile conditions, of 0.5 g of soya oil saccharose glyceride in 40 g of isopropanol is absorbed under sterile conditions on 199.5 g of sterile galactose particles, the isopropanol is removed by drying at 40°C and 200 torr and the microparticles are then ground in an air-jet mill until the following distribution of the particle size is obtained:

Median value: 1.9 µm

at least 99 % < 6 µm

at least 90 % < 3 µm.

The particle size and the distribution thereof are

determined in a particle-measuring apparatus, for example after suspension in isopropanol. 5 ml phials are each filled with 2 g portions of the microparticles.

C) For the preparation of 5 ml of the ultrasound contrast agent in a form ready for use, the contents of the phial containing carrier liquid (water for injection purposes, A) are introduced by means of an injection syringe into the phial containing microparticles (B) and shaken until a homogeneous suspension is formed (from 5 to 10 seconds).

Example 3

A) Preparation of the carrier liquid:

4.5 g of sodium chloride are dissolved in water to a volume of 500 ml and the solution is forced through a 0.2 µm filter; 5 ml phials are each filled with 4 ml of this solution and sterilised for 20 minutes at 120°C.

B) Preparation of the microparticles:

A solution, filtered under sterile conditions, of 0.5 g of polyethylene glycol sorbitan monostearate in 40 g of isopropanol is absorbed under sterile conditions on 199.5 g of sterile galactose particles, the isopropanol is removed by drying at 40° and 200 torr and the particles are then ground in an air-jet

mill until the following size distribution of the particle size is obtained:

Median value: 1.9 µm

at least 99 % < 6 µm

at least 90 % < 3 µm.

The particle size and the distribution thereof are determined in a particle-measuring apparatus, for example after suspension in isopropanol. 5 ml phials are each filled with 2 g portions of the micro-particles.

C) For the preparation of 5 ml of the ultrasound contrast-agent in a form ready for use, the contents of the phial containing carrier liquid (water for injection purposes, A) are introduced by means of an injection syringe into the phial containing micro-particles (B) and shaken until a homoceneous suspension is formed (from 5 to 10 seconds).

Example 4

A) Preparation of the carrier liquid:

4.5 g of sodium chloride are dissolved in water to a volume of 500 ml and the solution is forced through a 0.2 µm filter; 5 ml phials are each filled with 4 ml of this solution and sterilised for 20 minutes at 120°C.

B) Preparation of the microparticles:

A solution, filtered under sterile conditions, of 0.5 g of palm oil xylite in 40 g of isopropanol is absorbed under sterile conditions on 199.5 g of sterile galactose particles, the isopropanol is removed by drying at 40° and 200 torr and the particles are then ground in an air-jet mill until the following distribution of the particle size is obtained:

Median value: 1.9 µm

at least 99 % < 6 µm

at least 90 % < 3 µm.

The particle size and the distribution thereof are determined in a particle-measuring apparatus, for example after suspension in isopropanol. 5 ml phials are each filled with 2 g portions of the microparticles.

C) For the preparation of 5 ml of the ultrasound contrast agent in a form ready for use, the contents of the phial containing carrier liquid (water for injection purposes, A) are introduced by means of an injection syringe into the phial containing microparticles (B) and shaken until a homogeneous suspension is formed (from 5 to 10 seconds).

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A contrast agent for use in the ultrasound diagnosis of the human or animal body, which comprises in a liquid carrier a mixture of microparticles of a semi-solid or liquid surface-active substance and microparticles of a solid non-surface-active substance and micro-bubbles of a gas.

2. A contrast agent as claimed in claim 1, wherein the semi-solid or liquid surface-active substance is a lecithin, a polyoxyethylene fatty acid ester, a glycerine polyethylene glycol ricinoleate, a polyoxyethylene-polyoxypropylene polymer, a saccharose ester, a xyloglyceride, an unsaturated (C_4-C_{20})-fatty alcohol, an unsaturated (C_4-C_{20})-fatty acid, a mono-, or di- or tri-glyceride or a fatty acid ester, or a mixture of any two or more of such substances.

3. A contrast agent as claimed in claim 1 wherein the semi-solid or liquid surface-active substance is butyl stearate, soya oil saccharose glyceride or polyethylene glycol sorbitan monostearate, or a mixture of any two or more of such substances.

4. A contrast agent as claimed in claim 1 wherein the solid non-surface-active substance is a cyclodextrine, a monosaccharide, a disaccharide, a trisaccharide, a polyol or an inorganic or organic salt, or a mixture of any two or more of such substances.

5. A contrast agent as claimed in claim 1 wherein the solid non-surface-active substance is galactose, lactose or α-cyclodextrine, or a mixture of two or more of such substances.

6. A contrast agent as claimed in claim 1 wherein the microparticles of the semi-solid or liquid surface-active substance are present in a quantity of from 0.01 to 5% by weight.

7. A contrast agent as claimed in claim 6 wherein the microparticles of the semi-solid or liquid surface-active substance are present in a quantity of from 0.04 to 0.5%.

8. A contrast agent as claimed in claim 1 wherein the microparticles of the solid non-surface-active substance are present in a quantity of from 5 to 50% by weight.

9. A contrast agent as claimed in claim 8 wherein the microparticles of the solid non-surface-active substance are present in a quantity of from 9 to 40% by weight.

10. A contrast agent as claimed in claim 1 wherein the liquid carrier is water, a physiological electrolyte solution, an aqueous solution of a monohydric or polyhydric alcohol, or an aqueous solution of a mono- or di-saccharide.

11. A contrast agent as claimed in claim 10 wherein the liquid carrier is glycerine, polyethylene glycol or propylene glycol methyl ester.

12. A contrast agent as claimed in claim 10 wherein the liquid carrier is physiological sodium chloride solution.

13. A contrast agent as claimed in claim 1, 2 or 3 which contains microparticles of a mixture of butyl stearate and galactose in water.

14. A contrast agent as claimed in claim 1, 2 or 3 which contains microparticles of a mixture of soya oil saccharose glyceride and galactose in water.

15. A contrast agent as claimed in claim 1, 2 or 3 which contains microparticles of a mixture of polyethylene glycol sorbitan monostearate and galactose in physiological sodium chloride solution.

X

16. A contrast agent as claimed in claim 1, 2 or 3 which contains microparticles of a mixture of palm oil xylite and galactose in physiological sodium chloride solution.

17. A contrast agent as claimed in claim 1, 2 or 3 wherein the microparticles have a median particle size of from 1 to 3 μm.

18. A liquid medium for use in making up the contrast agent claimed in claim 1 which comprises a suspension of a mixture of microparticles of a semi-solid or liquid surface-active agent and microparticles of a solid non-surface active substance in a liquid carrier.

19. A liquid medium as claimed in claim 18 which contains from 0.01 to 5% by weight of the microparticles of the semi-solid or liquid surface-active agent.

20. A liquid medium as claimed in claim 19 which contains from 0.04 to 0.5% by weight of the microparticles of the semi-solid or liquid surface-active agent.

21. A liquid medium as claimed in claim 18 which contains from 5 to 50% by weight of the microparticles of the solid non-surface-active substance.

22. A liquid medium as claimed in claim 21 which contains from 9 to 40% by weight of the microparticles of the solid non-surface-active substance.

23. A liquid medium as claimed in claim 18 wherein the semi-solid or liquid surface-active substance is a substance(s) as claimed in claim 2 or 3.

24. A liquid medium as claimed in claim 18 wherein the solid non-surface-active substance is a substance(s) as claimed

in claim 4 or 5.

25. A liquid medium as claimed in claim 18 wherein the liquid carrier is a liquid as claimed in claim 10 or 11.

26. A liquid medium as claimed in claim 18, 19 or 20 wherein the microparticles have a median particle size of from 1 to 3 μm.

27. A diagnostic kit for use in the ultrasound diagnosis of the human or animal body, which comprises (A) a container which contains a liquid carrier, and (B) a second container which contains a mixture of microparticles of a semi-solid or liquid surface-active substance and microparticles of a solid non-surface-active substance.

28. A diagnostic kit as claimed in claim 27 wherein the containers (A) and (B) each have a volume of from 5 to 10 ml.

29. A diagnostic kit as claimed in claim 27 wherein the ratio by weight of the surface-active substance to the non-surface-active substance is from 0.01 to 5:100.

30. A diagnostic kit as claimed in claim 27 wherein the surface-active substance is a substance(s) as claimed in claim 2 or 3.

31. A diagnostic kit as claimed in claim 27 wherein the non-surface-active substance is a substance(s) as claimed in claim 4 or 5.

32. A diagnostic kit as claimed in claim 27 wherein the liquid carrier is a liquid as claimed in claim 10 or 11.

33. A diagnostic kit as claimed in claim 27, 28 or 29 wherein the microparticles have a median particle size of from 1

X

to 3 μm.

34. A diagnostic kit as claimed in claim 27, 28 or 29 which also comprises an injection syringe for transferring the contents of container (A) to container (B).

35. A diagnostic kit as claimed in claim 27, 28 or 29 wherein each of the containers (A) and (B) is a phial or an ampoule.

36. An ampoule or phial for use in ultrasound diagnosis of the human or animal body, which contains a contrast agent as claimed in claim 1, 2 or 3.

37. A process for the preparation of a contrast agent as claimed in claim 1, 2 or 3 wherein microparticles of a semi-solid or liquid surface-active substance and microparticles of a solid non-surface-active substance are mixed with a liquid carrier and agitated until a homogeneous suspension is formed.

*